

FORM PTO-1390 TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE ATTORNEY'S DOCKET NUMBER 51454-013
		U.S. APPLIC. NO. (if known, see 37 CFR 1.5) 10/049501
INTERNATIONAL APPLICATION NO PCT/AU00/00953	INTERNATIONAL FILING DATE August 11, 2000	PRIORITY DATE CLAIMED August 13, 1999
TITLE OF INVENTION FEED SUPPLEMENT FOR ALTERING MILK FAT PROFILE		
APPLICANTS FOR DO/EO/US Trevor William SCOTT, John Richard ASHES and Suresh Kumar GULATI		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.		
1.	<input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U S C. 371.	
2.	<input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U S C. 371	
3.	<input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1)	
4.	<input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.	
5.	<input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau (The Published International Application is enclosed herewith.) c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)	
6.	<input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2))	
7.	<input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau) b. <input checked="" type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made, however, the time limit for making such amendment has NOT expired. d. <input type="checkbox"/> have not been made and will not be made.	
8.	<input checked="" type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U S C. 371(c)(3)).	
9.	<input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U S C. 371(c)(4)).	
10.	<input checked="" type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))	
Items 11. to 16. below concern other document(s) or information included:		
11.	<input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98	
12.	<input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included	
13.	<input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment	
14.	<input type="checkbox"/> A substitute specification	
15.	<input type="checkbox"/> A change of power of attorney and/or address letter.	
16.	<input checked="" type="checkbox"/> Other items or information 1. International Search Report prepared by Australian Patent Office 2. International Preliminary Examination Report. 3. PCT/IB/308 4. Amended claims under Article 19.	



20277

PATENT TRADEMARK OFFICE

JC13 Rec'd PCT/PTO 13 FEB 2002

U.S. APPLIC. NO. (if known, see 37 CFR 1.50) 10/049501	INTERNATIONAL APPLICATION NO. PCT/AU00/00953	ATTORNEY'S DOCKET NUMBER 51454-013		
		CALCULATIONS PTO USE ONLY		
17. <input checked="" type="checkbox"/> The following fees are submitted:				
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO		\$890.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))		\$710.00 \$740.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO		\$1,040.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)		\$100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$1,040.00		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$130.00		
Claims	Number Filed	Number Extra	Rate	
Total Claims	22 -20 =	2	x \$18.00	\$36.00
Independent Claims	2 -3 =		x \$84.00	\$0.00
Multiple dependent claim(s) (if applicable)		+ \$280.00		\$280.00
TOTAL OF ABOVE CALCULATIONS =			\$1,486.00	
Reduction by 1/2 for filing by small entity, if applicable Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).			\$0.00	
SUBTOTAL =			\$1,486.00	
Processing fee of \$130.00 for furnishing the English translation later than the <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		+ \$0.00		
TOTAL NATIONAL FEE =			\$1,486.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property		+ \$0.00		
TOTAL FEES ENCLOSED =			\$1,486.00	
		Amount to be, refunded	\$	
		charged	\$1,486.00	

- a. A check in the amount of \$ _____ to cover the above fees is enclosed
- b. Please charge my Deposit Account No. 500417 in the amount of \$ 1,486.00 to cover the above fees A duplicate copy of this sheet is enclosed.
- c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500417. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO.

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REGISTRATION NUMBER	
February 13, 2002	

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Docket No.: 51454-013

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :
Trevor William SCOTT, et al. :
Serial No.: Group Art Unit:
Filed: February 13, 2002 : Examiner:
For: FEED SUPPLEMENT FOR ALTERING MILK FAT PROFILE

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE CLAIMS:

Please replace claims 1-21, as originally filed with claims 1-21 as amended under PCT Article 19.

REMARKS

The original claims have been replaced with the claims as amended under PCT Article 19. Attached hereto is a clean copy of the claims as amended.

Entry of this preliminary amendment is respectfully requested.

Respectfully submitted,

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Feed Supplement for Altering Milk Fat Profile**Technical Field**

The present invention relates to feeding techniques for designing the nutritional and physico-chemical properties of milk fat derived from ruminants. In particular, it 5 describes feed supplements which produce milk from ruminants having a desired fatty acid composition, and which is useful in producing products with a range of melting profiles.

Background Art

In recent times, the specifications of the ideal milk fat from a nutritional and 10 physico-chemical view point have changed dramatically. For example, C18 *cis* monounsaturated fatty acids (oleic acid) have been shown to lower the cholesterol content of human low density lipoproteins (LDL) (Noakes *et al.*, 1996). In contrast, the C18 *trans* monounsaturated fatty acid (elaidic acid) will increase the cholesterol content of LDL in 15 humans (Noakes *et al.*, 1996). In addition, the role of n-3 fatty acids in infant nutrition and in particular their importance in neural development and vision has been recently recognised (Simopoulos, 1999).

During the past three decades a range of feed supplements have been developed 20 with the aim of manipulating the fatty acid composition of milk fat. These techniques include feeding of full fat rape seed and soybean supplements, heat treated/jet sploded oil seeds, calcium salts of long chain fatty acids, prilled or pelleted fats and butyl soyamide esters. However, there is an enormous variation in the responses observed (Palmquist, *et* 25 *al.*, 1993), and it can be concluded that these approaches do not provide a reliable and consistent feed supplement to alter the nutritional and physico-chemical properties of milk fat.

25 Therefore, the challenge is to design feed supplements that produce milk fat containing a fatty acid composition appropriate for either soft or hard fats. For example, soft fats would be characterised by:

- * a reduction in the proportions of saturated acids in particular myristic and palmitic, as these two acids significantly elevate human LDL cholesterol and also contribute to "hardness" of milk fat
- * an increase in C18 *cis* mono-unsaturated (oleic) without increasing C18 *trans* mono-unsaturated (elaidic);
- * an increase in C18 di-unsaturated (C18:2), including conjugated isomers;

* an increase in C20 and C22 omega fatty acids, that is, C20:5 and C22:6 respectively; and

* an increase in C18 tri-unsaturated (C18:3).

Conversely, harder milk fats are often characterised by:

5 * high proportions of saturated fats; and
* increases in C16:0 and C18:0.

Therefore, in accordance with the present invention, by altering the amount and/or type of protected lipid fed, it is possible to produce ruminant milk products with a wide spectrum of physical characteristics. Consequently, the present invention provides a 10 way forward to reduce or eliminate the need for expensive fractional crystallisation and enzymatic inter-esterification procedures that are currently being used to improve the physical and nutritional properties of milk fat. In general, the present invention indicates alters the fatty acid profile of ruminant milk fat via the use of feed supplements in which the constituent triacylglycerols are protected from ruminal biohydrogenation

15 Accordingly, the present invention describes the use of nutritional materials that are protected against rumen degradation and provides a feed supplement which produces milk fat with the desired specifications, that is, a milk fat having either a "soft" or "hard" fatty acid profile.

Object of the Invention

20 An object of the invention is to provide a method for altering the fatty acid profile of milk from ruminant livestock, and in doing so obtain milk fat comprising desired proportions and/or types of fatty acids.

Disclosure of the Invention

According to a first embodiment of the invention there is provided a method for 25 altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the female ruminant livestock protected lipid having said desired proportions and/or types of fatty acids, such that about 60 to about 90% of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90% of said protected lipid 30 available for digestion post-ruminally.

According to a second embodiment of the invention there is provided a protected lipid, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected lipid is such that about 60 to about 90% of said protected lipid is capable of passing through the rumen

of ruminant livestock undigested, leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

According to a third embodiment of the invention there is provided use of a protected lipid, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected lipid is such that about 60 to about 90% of said protected lipid is capable of passing through the rumen of ruminant livestock undigested, leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally.

It is preferred that about 65 to about 90% of protected lipids are capable of passing undegraded through the rumen. More preferably, about 70 to about 90% of protected lipids are capable of passing undegraded through the rumen. Even more preferably, about 72 to about 90% of protected lipids are capable of passing undegraded through the rumen. Yet still more preferably, about 75 to about 90% of protected lipids are capable of passing undegraded through the rumen.

Typically, the protected lipid is protected from ruminal biohydrogenation by encapsulation in a matrix of aldehyde-treated protein.

According to a fourth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the female ruminant livestock, protected lipid having said desired proportions and/or types of fatty acids, wherein said protected is lipid produced by the emulsification of lipid with protein in the presence of between about 1.5 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

According to a fifth embodiment of the invention there is provided a protected lipid having desired proportions and/or types of fatty acids, when used in altering the fatty acid profile of milk from female ruminant livestock to have said desired proportions and/or types of fatty acids, wherein said protected is lipid produced by the emulsification of lipid with protein in the presence of between about 1.5 grams to about 3.0 grams of formaldehyde per 100 grams crude portion.

According to a sixth embodiment of the invention there is provided use of a protected lipid having desired proportions and/or types of fatty acids, in the preparation of feed for altering the fatty acid profile of milk from female ruminant livestock to have said desired proportions and/or types of fatty acids, wherein said protected lipid is produced by the emulsification of lipid with protein in the presence of between about 1.0 grams to about 3.5 grams of formaldehyde per 100 grams crude portion.

It is preferred that the protected lipid is produced by the emulsification of lipid with protein in the presence of between about 1.75 grams to about 3.0 grams of formaldehyde per 100 grams crude portion. Even more preferably, about 2.0 grams to about 3.0 grams of formaldehyde per 100 grams crude portion. Still more preferably, 5 about 2.0 grams to about 2.8 grams of formaldehyde per 100 grams crude portion. Yet still more preferably, about 2.0 grams to 2.6 grams of formaldehyde per 100 grams crude portion.

Preferably, the protected lipid fed in accordance with any one of the first through to sixth embodiments of the invention does not constitute the entire ration, but may be fed 10 together with any other source of processed or unprocessed feedstuff.

Typically, the ruminant livestock fed the protected lipid in accordance with the present invention are selected from the group consisting of: cattle, sheep, goats and buffalo.

Typically, the term "fatty acid profile" describes the particular fatty acid 15 constituents of milk obtained from female ruminant livestock fed protected lipid comprising the particular fatty acid constituents to obtain the desired fatty acid profile.

In one aspect, a preferred fatty acid profile may reflect milk fat containing a high proportion of soft fats. Typically, such a softer fatty acid profile is a consequence of a milk fat containing less saturated and more unsaturated fatty acids (desired proportions of fatty acids). More typically, these fats are characterised by any one of the following: reduction in the proportions of saturated acids in particular myristic and palmitic, as these two acids significantly elevate human LDL cholesterol and also contribute to the "hardness" of milk fat; an increase in C18 *cis* mono-unsaturated (oleic) fatty acids without increasing C18 *trans* mono-unsaturated (elaidic) fatty acids; an increase in C18 di-unsaturated (C18:2) fatty acid, including conjugated forms of linoleic acid; an increase in C18 tri-unsaturated (C18:3) fatty acid; and/or an increase in C20 and C22 omega unsaturated fatty acids, such as, C20:5 and/or C22:6.

A milk fat reflecting a softer fatty acid profile may typically be produced by feeding female ruminant livestock a protected lipid source containing C18 monounsaturated or polyunsaturated fats, or lipids high in C20 or C22 polyunsaturated fatty acids, such as C22:5 and/or C22:6 fatty acids. More typically, the protected lipid source is a oleyl, linoleyl or linolenyl oil containing oil seed.

Typically, the protected lipid source fed to obtain such a softer milk fatty acid profile is selected from the group consisting of plant derived materials including canola oilseed, soybean oilseed, sunflower oilseed, linseed (flax) oilseed, sesame oilseed, grape

oilseed, olive oilseed, safflower oilseed, groundnut oilseed, oils derived from these seeds and oil by products (ie, acid oil or conjugated linoleic acid) produced during refining/hydrogenation processes, marine sources, such as fish oils or mixtures thereof, and oils produced by either chemical, microbiological or biotechnology procedures and alkali isomerisation techniques.

In a preferred aspect, the present invention provides a method for producing softer milk fat which comprises the feeding of canola/soybean oilseed supplement in ratios of about 7:3 (w/w) protected from ruminal degradation. Still more preferably, the present invention provides a method for producing softer milk fat which comprises the feeding of canola/soybean oilseed supplement in ratios of about 7:3 (w/w), soybean oilseed/linseed of about 7:3 (w/w) and soybean oilseed/Holeic sunflower oilseed of about 7:3,(w/w) and soybean oilseed/fishoil of about 7:3 (w/w), wherein these lipid sources are protected from ruminal degradation.

Typically, the protected lipid as fed, and as a consequence, the fatty acid profile of milk so produced, comprises the following proportions of fatty acids: C18:1 cis (25-45%w/w), C18:2 (4-15%w/w) and C18:3 (1-8%w/w). Still more typically, the protected lipid as fed comprises the following proportions of fatty acids: C18:1 cis (30-40%w/w), C18:2 (6-10%w/w), including conjugated isomers (0.5 to 5%), C18:3 (1-4%w/w) and C20 and C22 omega fatty acids, C20:5 and C22:6, (1-2%w/w).

In another aspect of the invention, the desired proportions and/or types of fatty acids in the altered fatty acid profile of the milk reflect a milk fat having a harder fatty acid profile, wherein the harder fatty acid profile is a consequence of a milk fat comprising more saturated and less unsaturated fatty acids, which is produced by feeding female ruminant livestock protected lipid comprising more saturated and less unsaturated fatty acids. Typically, the protected lipid source fed to obtain a harder milk fatty acid profile is high in hydrogenated fats. Even more typically, such fats are characterised by: high proportions of saturated fats and increases in the relative proportions C16:0 and C18:0 fatty acids.

Typically, the protected lipid source fed to obtain a harder milk fatty acid profile is selected from the group consisting of: cotton oilseed, palm oilseed, tallow, lard and sources derived from hydrogenated or partially hydrogenated processes or produced by either chemical, microbiological and biotechnology procedures or mixtures thereof; or other naturally occurring sources of oils/oilseeds that contain inhibitors of the desaturase enzyme systems which operate in ruminant tissues, wherein examples of these inhibitors include cyclopropenoids such as sterculate.

In a preferred aspect, the present invention provides a method for producing hard milk fat containing more C16:0 and C18:0 saturated and less unsaturated fatty acids. Such a profile arises from the feeding of cotton oilseed supplement or cotton oilseed and palm oilseed in ratios of about 8:2 (w/w), but more preferably, 4:2 (w/w), protected from 5 ruminal degradation.

Typically, the protected lipid as fed, and as a consequence, the fatty acid profile of milk so produced, comprises the following proportions of fatty acids: 25-35%w/w C16:0, 20-30%w/w C18:0 and 20-25%w/w C18:1. Still more typically, 28-35%w/w C16:0, 25-30%w/w C18:0 and 22-25%w/w C18:1. Yet still more typically, 30-35%w/w 10 C16:0 and 25-30% C18:0%w/w.

In general, the protected lipid is as described in Australian Patent Nos. 450 530 and 659 557, the disclosures of which are incorporated herein by reference.

According to a seventh embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired 15 proportions and/or types of fatty acids, wherein said method is in accordance with the first or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for 20 digestion post-ruminally.

According to an eighth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected protein, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such 25 that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

According to a ninth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further 30 comprising protected protein, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally.

More typically, about 65 to about 80% of protected protein is capable of passing undegraded through the rumen. Even more typically, about 70 to about 80% of protected protein is capable of passing undegraded through the rumen. Still more typically, about 72 to about 80% of protected protein is capable of passing undegraded through the rumen.

5 Yet still more typically, about 75 to about 80% of protected protein is capable of passing undegraded through the rumen.

According to a tenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method is in accordance with the first 10 or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock protected protein, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein.

According to an eleventh embodiment of the invention there is provided 15 protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected protein, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein.

According to a twelfth embodiment of the invention there is provided use of 20 protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected protein, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein.

Typically, the protected protein is produced by the reaction with between about 25 0.1g and about 1.0g of formaldehyde per 100g crude protein. More typically, the protected protein is produced by the reaction with between about 0.15g and about 1.0g of formaldehyde per 100g crude protein. Even more typically, the protected protein is produced by the reaction with between about 0.2g and about 1.0g of formaldehyde per 100g crude protein. Still more typically, the protected protein is produced by the reaction with between about 0.2g and about 0.9g of formaldehyde per 100g crude protein.

In general, the protected protein is as described in Australian Patent No. 659 557, the disclosure of which is incorporated herein by reference.

Preferably, the protected lipid and protein fed in accordance with any one of the seventh through to twelfth embodiments of the invention does not constitute the entire ration, but may be fed together with any other source of processed or unprocessed feedstuff.

According to a thirteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method is in accordance with the first or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a fourteenth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a fifteenth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

It is preferred that about 40 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. More preferably, about 50 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. Still more preferably, about 60 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. Even still more typically, about 65 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen.

According to a sixteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method is in accordance with the first

or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock protected carbohydrate, wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate.

According to a seventeenth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising protected carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate.

According to an eighteenth embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising protected carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate.

It is preferred that the protected carbohydrate is produced by the reaction with between about 0.1 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate. More preferably, the protected carbohydrate is produced by the reaction with between about 0.5 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate. Even more preferably, the protected carbohydrate is produced by the reaction with between about 1.0 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate. Still more preferably, the protected carbohydrate is produced by the reaction with between about 1.5 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate.

Preferably, the protected lipid and carbohydrate fed in accordance with any one of the thirteenth through to eighteenth embodiments of the invention does not constitute the entire ration, but may be fed together with any other source of processed or unprocessed feedstuff.

According to a nineteenth embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method is in accordance with the first or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock (i) protected protein,

such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

According to a twentieth embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-first embodiment of the invention there is provided use of a protected lipid in accordance with the third or sixth embodiments of the invention, further comprising (i) protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

Typically, (i) about 65 to about 80% of protected protein is capable of passing undegraded through the rumen, and (ii) 40 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. More typically, (i) about 70 to about 80% of protected protein is capable of passing undegraded through the rumen, and (ii) 50 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. Still more typically, (i) about 72 to about 80% of protected protein is capable of passing undegraded through the rumen, and (ii) about 60 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen. Yet still more typically, (i) about 75 to about 80% of protected protein is capable of passing undegraded

through the rumen, and (ii) about 65 to about 80% of protected carbohydrates are capable of passing undegraded through the rumen.

According to a twenty-second embodiment of the invention there is provided a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method is in accordance with the first or fourth embodiments of the invention, and wherein said method further comprises simultaneously feeding to the female ruminant livestock (i) protected protein, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, and wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate.

According to a twenty-third embodiment of the invention there is provided protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, and wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate, when used in altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

According to a twenty-fourth embodiment of the invention there is provided use of a protected lipid in accordance with the second or fifth embodiments of the invention, further comprising (i) protected protein, wherein said protected protein is produced by the reaction with between about 0.05g and about 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate, wherein said protected carbohydrate is produced by the reaction with between about 0.1 grams and about 3 grams of formaldehyde per 100 grams carbohydrate, in the preparation of a feed for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids.

Typically, (i) the protected protein is produced by the reaction with between about 0.1g and about 1.0g of formaldehyde per 100g crude protein, and (ii) the protected carbohydrate is produced by the reaction with between about 0.1 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate. More typically, (i) the protected protein is produced by the reaction with between about 0.15g and about 1.0g of formaldehyde per 100g crude protein, and (ii) the protected carbohydrate is produced by the reaction with between about 0.5 grams and about 2.5 grams of formaldehyde per 100

grams carbohydrate. Even more typically, (i) the protected protein is produced by the reaction with between about 0.2g and about 1.0g of formaldehyde per 100g crude protein, and (ii) protected carbohydrate is produced by the reaction with between about 1.0 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate. More typically, (i) the 5 protected protein is produced by the reaction with between about 0.2g and about 0.9g of formaldehyde per 100g crude protein, and (ii) the protected carbohydrate is produced by the reaction with between about 1.5 grams and about 2.5 grams of formaldehyde per 100 grams carbohydrate.

Preferably, the protected lipid, protein and carbohydrate fed in accordance with 10 any one of the nineteenth through to twenty-fourth embodiments of the invention does not constitute the entire ration, but may be fed together with any other source of processed or unprocessed feedstuff.

According to a twenty-fifth embodiment of the invention there is provided a milk 15 fat obtained from a female ruminant animal fed in accordance with the method of any one of the first, fourth, seventh, tenth, thirteenth, sixteenth, nineteenth or twenty-second embodiments of the invention, or obtained from a female ruminant animal fed a protected lipid, the lipid in accordance with the second, fifth, eighth, eleventh, fourteenth, seventeenth, twentieth or twenty-third embodiments of the invention, or obtained from a female ruminant animal fed a feed prepared in accordance with the use of any one of the 20 third, sixth, ninth, twelfth, fifteenth, eighteenth, twenty-second or twenty-fourth embodiments of the invention.

Typically, the milk fat in accordance with the twenty-fifth embodiment of the 25 invention is either a soft or hard fat. More typically, the milk fat is comprised of nutritionally desirable soft fats, including n-3 and n-6 essential fatty acids, conjugated linoleic acid and C₂₀ and C₂₂ polyenoic fatty acids.

Typically, the milk fat obtained in accordance with the twenty-fifth embodiment 30 of the invention is used in the production of milk based products. More typically, the milk based products may be selected from the group consisting of: milk, butter, cheese, yoghurt, chocolate and infant formula. Even more typically, the milk based product is butter having an altered spreadability.

Brief Description of the Drawings

Figure 1 illustrates a graphic representation of the role of feedstuffs, including 2 protected lipids, in altering the proportions of fatty acids in milk. Figure 1 illustrates the differences in melting profiles between softer milk fats produced from cows receiving

kg and 3 kg of protected canola/soybean (7:3 w/w) supplement per day and normal milk fats derived from cows grazing pasture, together with a polyunsaturated margarine for comparison.

Figure 2 also illustrates a graphic representation of the role of protected lipids, in altering the proportions of fatty acids in milk, in this case, producing harder milk fats. Figure 2 reflects an increase in the proportion of C18:0 and a decrease in C18:1, thereby resulting in a substantial increase in both the melting point of milk fat and its hardness, and reference is made to Example 7 for the feeding regime which results in this fatty acid profile.

10

Definitions

In the context of the present invention, the following terms have the meanings set out below.

15

In this specification the term "simultaneously" is used to mean feeding of the ruminant livestock within a period of about 24 hours, that is, to realise the benefits of any one of the seventh through to twenty-fourth embodiments of the invention it is not essential that the intake of protected lipid and protected protein, and/or protected carbohydrate takes place at the same time, rather it is important that within a given 24 hour period the animals blood plasma is enriched with lipid, protein and/or carbohydrate constituents by absorption from the abomasum or lower digestive tract.

20
25
30

By "protected" we mean treated so as not to be fully exposed to the degradative action of the ruminant environment, but available for absorption from the abomasum or lower digestive tract. Lipids are protected by their encapsulation in a matrix of aldehyde treated protein. Importantly, the degree of protection of the formaldehyde-treated protein encapsulating the lipid is much greater than the degree of protection afforded the encapsulating protein alone. That is, the availability of the encapsulating protein protecting the lipid is sacrificed to a large extent in order to maintain the lipid beyond the rumen. Thus ensuring that almost all the protected lipid does indeed pass through the rumen undigested. For the purposes of this invention dietary lipids can be protected from ruminal metabolism by encapsulation in such a matrix of cross-linked proteins, and the preferred window of protection ranges from 60% to 90%. In terms of the protected protein constituent of feed of the invention, the degree of rumen protection lies in the range 60 to 80%, that is, 60 to 80% of the protein content of the supplement will pass undegraded through the rumen. Similarly, in terms of the protected carbohydrate constituent of feed of the invention, the degree of rumen protection lies in the range 30 to

80%, that is, 30 to 80% of the carbohydrate content of the supplement will pass undegraded through the rumen.

Suitable techniques should allow accurate control of the amount of cross-linking that occurs between the lipid, protein and carbohydrate feedstuffs, and the aldehyde. This
5 may be achieved by varying the amount of aldehyde relative to the lipid, protein and carbohydrate content, so that the lipid, protein and carbohydrate is optimally "protected" from rumen degradation, but may be completely digested and absorbed from the small intestine.

"Protected lipid" is defined as lipid soluble material that normally contains long
10 chain fatty acids and is treated either chemically or physically to reduce its degradation in the rumen, but allows the fatty acids to be available for absorption from the intestine. The degree of protection ranges from about 60 to about 90%, that is, about 60 to about 90% of the fat supplement will pass undegraded through the rumen. In the context of the present invention when protected lipid is fed, a degree of protection of about 75 to about 90% is
15 preferred.

"Protected protein" is defined as proteinaceous material that is treated chemically or physically to reduce the rate of degradation of the constituent amino acids in the rumen. The degree of protection will vary from about 60 to about 80%, that is, about 60 to about 80% of the protein will pass undegraded through the rumen. In the context of the present
20 invention when protected protein is fed, a degree of protection of about 70-75 to about 80%, is preferred.

"Protected carbohydrate" is defined as carbohydrates or carbohydrate containing material that is treated chemically or physically to reduce the rate of degradation in the rumen but allows the carbohydrate to be readily digested in the small intestine. The degree
25 of protection will vary from about 30 to about 80%, that is, about 30 to about 80% of the carbohydrate will pass undegraded through the rumen. In the context of the present invention when protected carbohydrate is fed, a degree of protection of about 65 to about 80% is preferred.

By "grain" we mean plant derived concentrates, and these include barley, wheat,
30 oats, sorghum etc.

By "carbohydrate" we mean complex carbohydrates such as polyhydroxy aldehydes, ketones, alcohols or acids, their derivatives, and any compound that may be hydrolysed to these.

"Protein" is defined as proteinaceous material containing individual amino acids
35 linked together.

"Fat" is defined as lipid soluble material and normally contains long chain fatty acids of carbon chain length >C10.

By "roughage" we mean plant derived cellulose materials containing varying proportions of fibre which are digested at different rates in the rumen.

5 By "minerals and vitamins" we mean supplement of anions, cations, trace elements and fat-soluble vitamins A, C, D and E that are normally included in feed rations.

Best Modes of Carrying Out the Invention

In the performance of this invention in general, protected lipid is included in the 10 ration in an amount up to about 45% of dry matter intake. More preferably, protected lipid is included in the ration in an amount between about 10% to about 30% of dry matter intake. Even more preferably, in an amount between about 8% to about 16% of dry matter intake. Still more preferably, in an amount between about 8% to about 12% of dry matter intake.

15 However, it is likely to be most practical to feed animals protected lipid as a supplement which also combines both a protected protein and a protected carbohydrate. In those instances where protected lipids are used in combination with protected carbohydrate and/or protected protein, a ratio of 1:1:1 w/w/w is often used to manufacture the protected feed supplement, and the supplement is typically included in the ration at 20 about 10-45% during the lactation phase. Preferably the protected feed supplement is included in the ration at about 15-45% of dry matter intake during the lactation phase, more preferably, at about 15-30% of dry matter intake during the lactation phase, and even more preferably, at about 20-30% of dry matter intake during the lactation phase.

25 Preferably, the protected feed supplements are fed at a rate of between about 3 and about 5 kilograms per ruminant animal per day. More preferably, the protected feed supplements are fed at a rate of between about 4 and about 5 kilograms per ruminant animal per day.

30 Examples of the mechanisms by which protected lipid, protected protein and protected carbohydrate may be produced are described in Examples 8, 9 and 10 respectively.

An economically viable source of lipid, carbohydrate and protein is likely to be cereal grain. Sources of such cereal grain are likely to include: barley, maize, oats, wheat, rice, millet, triticale, rye, and sorghum. Other sources of lipid, carbohydrate and protein include oil seed, oil and lipids, derived from plants, animals and the by-products of food

processing for human consumption. As described by Kirk-Othmer (1980), sources of such oilseeds, oil and lipids include the following: corn, soybean, cotton, lupin, peanut, sunflower, canola, sesame seed oil, olive oil, copra and coconut oil, palm kernels and palm oil, casein, butterfat, lard, fish oils, linseed and oil, tung oil, tallow and yellow grease. A still further source of lipid includes lipid products or conjugated linoleic acid products, derived from oil sources via chemical, microbiological or biotechnology processes, including, alkali isomerisation techniques, or mixtures thereof; or other naturally occurring sources of oils/oilseeds that contain inhibitors of the desaturase enzyme systems which operate in ruminant tissues, wherein examples of these inhibitors include cyclopropinoids such as sterculate.

The wide diversity of lipid sources offers the flexibility to select components of the lipid according to the relative prices and availability of raw materials, and the same holds for carbohydrate or protein sources. The selection of the source of the lipid, carbohydrate and/or protein to be protected, is normally dependent on their seasonal availability and price. There is no particular inherent advantage provided by feeding any one lipid, nor for that matter, any one carbohydrate or protein source which precludes its use over another, provided of course that the source of lipid is such that it produces the desired proportions of fatty acids in the milk products.

Clearly the benefits possible from practising this invention can be expected to be related to the continuity and period of feeding the protected lipid and to amounts fed, but other factors such as animal specifications, eg. genotype, age, and physiological condition and the environmental situation (temperature, humidity), should also be taken into account when deciding on the feeding regime to be adopted.

In one aspect of the invention, softer milk fats may be obtained through the feeding of protected canola seed, sunflower seed, or any other oleyl or linoleyl oil containing oil seed, that is fats containing C18 monounsaturated or polyunsaturated fats. For example, lipids high in C18:1, C18:2 and C18:3 fatty acids.

Furthermore, the softer milk fats may be obtained through the feeding of protected fish oils. For example, lipids high in C20 or C22 polyunsaturated fatty acids, such as C22:5 and/or C22:6 fatty acids.

In a more preferred aspect, the present invention provides a method for producing softer milk fat containing less saturated and more unsaturated fatty acids, which comprises the feeding of canola/soybean oilseed supplement in ratios of about 7:3 (w/w) protected from ruminal degradation.

Preferably, the softer milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: C18:1 cis (25-45%w/w), C18:2 (4-15%w/w) and C18:3 (1-8%w/w). Even more preferably, the softer milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: C18:1 cis (30-40%w/w); C18:2 (6-10%w/w), including proportions (0.5 to 5%) of conjugated isomers, C18:3 (2-4%w/w); C20:5 and/or C22:6 (1-2% w/w).

In another aspect of the invention, there is provided a method for producing harder milk fat containing more saturated and less unsaturated fatty acids, which comprises for example the feeding of cotton oilseed supplements protected from ruminal degradation.

In another aspect of the invention, the harder milk fats may be obtained through the feeding of protected oils enriched in saturates, for example hydrogenated fats.

Preferably, the harder milk fats may be obtained through the feeding of protected cotton seed, due to the presence of cyclopropene fatty acids and additional dietary C18:2 which acts to inhibit $\Delta 9$ desaturase enzyme, an enzyme which converts additional C18:0 into C18:1 within the mammary gland.

Preferably, the harder milk fat obtained via the feeding regime of the present invention may contain the following proportions of fatty acids: 25-35%w/w C16:0, 20-30%w/w C18:0 and 20-25%w/w C18:1. More preferably, 28-35%w/w C16:0, 25-30%w/w C18:0 and 22-25%w/w C18:1, and still more preferably, 0-35%w/w C16:0 and 25-30% C18:0%w/w.

The milk fat produced by the feeding regime of the present invention may be used in all milk based products, including for example: milk, butter, cheese, yoghurt, chocolate and infant formulas.

Milk based products with the fatty acid characteristics obtained through the feeding regime of the present invention, such as for example: butter, cheese, yoghurt, chocolate and infant formulas, are produced according to the relevant manufacturing processes well accepted in the art.

Preferably, butter derived from the softer milk fat produced by the feeding regime of the present invention provides improved spreadability.

Preferably, milk based products with the fatty acid characteristics obtained through the feeding regime of the present invention contain a desirable ratio of n-6/n-3 fatty acids for human nutrition. More preferably, a desirable ratio of n-3/n-3 fatty acids is considered to be 5:1 or less. For example, in Table 2, a ratio of 3.1:1 was achieved by

feeding protected canola soybean supplements to dairy cows at the rate of approximately 2.5 kg per head per day, equivalent to 750g fat (see Table 1).

In accordance with the invention, the feeding of protected lipid, together with protected protein and protected carbohydrate, in addition to designing milk fat profiles, 5 also results in improvements in relation to growth rate and/or carcass quality.

Test Methods

1. *In-Vitro* Biological Evaluation of Feed Supplements

(a) Ruminal hydrogenation of unsaturated lipids.

Samples of unsaturated lipid supplements (containing ca. 40-50mg of oil) are 10 incubated in test tubes with 10mL of strained rumen fluid. The tubes are flushed with nitrogen, capped with rubber serum caps and incubated in a shaking water bath at 38°C for periods up to 20h. The incubated and corresponding unincubated reaction mixtures are saponified and the fatty acids extracted and methylated. The methyl esters are analysed by gas liquid chromatography (GLC), and the extent of protection against 15 ruminal hydrogenation calculated using the formula:

$$\text{Protection (\%)} = \frac{\% \text{ 18:2 after incubation}}{\% \text{ 18:2 before incubation}} \times 100$$

The endogenous level of polyunsaturated fatty acids in the rumen fluid was always less than 2% by weight of the total fatty acid, and thus had little effect on the above calculations. The hydrogenating capacity of each batch of rumen fluid is verified 20 by incubating the rumen fluid with samples of polyunsaturated oil-casein supplements prepared without formalin.

(b) Ruminal lipolysis of triacylglycerol

Samples of the lipid supplements (containing ca. 40-50mg of lipid) are incubated with 10mL of strained rumen fluid as described above. When the extent of triacylglycerol 25 (TG) hydrolysis is measured by GLC, heptadecanoic acid (17:0)(20mg) is added to each reaction tube as an internal standard.

The incubated and corresponding unincubated reaction mixtures are extracted with 10mL of chloroform-methanol (C/M 2:1 v/v) containing 0.5mL of 5M HCl. The mixtures of rumen fluid and acidic C/M are vigorously shaken and allowed to stand for 2- 30 4h until two phases were clearly distinguished.

The upper aqueous phase is removed and discarded and the lower organic phase filtered to remove suspended matter. The filtrate is evaporated to dryness using rotary

film evaporator, and the extent of TG hydrolysis estimated using either thin layer chromatography (TLC), or if 17:0 was added, GLC methods described below.

(i) TLC analysis of the extracted lipids is carried out using silica gel G and a solvent system of petroleum ether: diethyl ether:acetic acid (84:15:1, v/v/v). The separated lipids are visualised by spraying with an ethanolic solution of 2,7-dichlorofluorescein (0.2% w/v) and viewing under UV light. The extent of TG hydrolysis can only be estimated qualitatively by comparing the relative intensities and sizes of the TG and free fatty acid (FFA) spots in both the incubated and the unincubated reaction mixtures.

(ii) GLC analysis is used in conjunction with the 17:0 internal standard to assess the degree of TG lipolysis. This method relies on the determination of the proportion of 17:0 in the FFA fraction of the incubated and the unincubated lipid extracts. The dilution of 17:0 in the FFA fraction which occurs during incubation is used as an index of ruminal lipolysis. The FFA in the lipid extracts are methylated with diazomethane and the methyl esters separated by GLC. In addition, samples of the total lipid extracts are saponified, acidified, and extracted with petroleum ether, and the total fatty acids obtained are also methylated with diazomethane and analysed by GLC. The GLC 17:0 measurements were used to estimate the following values:

TFA t_0 = Total fatty acids at 0h

TFA t_{20} = Total fatty acids at 20h

FFA t_0 = Free fatty acids at 0h

FFA t_{20} = Free fatty acids at 20h

EFFA t_0 = Endogenous ruminal free fatty acids at 0h (from unincubated rumen fluid controls)

EFFA t_{20} = Endogenous ruminal free fatty acids at 20h (from incubated rumen fluid controls).

From these values it was possible to calculate the following two other values:

RFA t_0 (released fatty acids at 0h) = FFA t_0 - EFFA t_0

and

RFA t_{20} (released fatty acids at 20h) = FFA t_{20} - EFFA t_{20}

The resistance to ruminal lipolysis is then calculated using the formula:

$$\text{Resistance (\%)} = \frac{\text{TFA } t_{20} - \text{RFA } t_{20}}{\text{TFA } t_0 - \text{RFA } t_0} \times 100$$

(c) Ruminal carbohydrate protection

The protection of carbohydrate is determined by the measurement of the residual starch remaining after 24h *in sacco*. 5g of treated or untreated carbohydrate are sealed into 3x5cm nylon bags (52 μ m pore size) which are inserted with appropriate weights in the rumen of a sheep for 24h. These bags are removed, washed in deionised water and freeze dried and the weight of residue remaining determined. The residues and incubated samples are ground through a mill (containing a 0.5mm screen) and the starch determined on a 100mg sub-samples enzymatically using a "Megazyme" total starch assay kit (distributed by Deltagen Australia, 31 Wadhurst Drive, Boronia, Victoria Australia, 3155). All starch values measured are corrected to known standards provided in the kit. The protection of the protected carbohydrate is then calculated as the ratio of the total starch in the untreated and treated sample.

(d) Ruminal protein solubility

The release of ammonia during *in vitro* incubation with rumen fluid is used as a measure of the solubility of the proteins. To 10mL of strained rumen fluid, sufficient lipid supplement is added to supply 75mg of protein, and the mixture was incubated anaerobically at 37°C for 20h. The reaction flasks including rumen fluid blanks are treated with 5mL of 0.2 M H₂SO₄. The mixtures are centrifuged to remove suspended matter, and ammonia is estimated in the supernatant after steam distillations. Net ammonia production is calculated from the difference between the incubated and blank values corrected for ammonia initially present.

2. *In-vivo* Biological Evaluation of Supplements**(a) Ruminal carbohydrate protection**

The protection of carbohydrate is determined by the measurement of the residual starch remaining after 24h *in sacco*. 5g of treated or untreated carbohydrate are sealed into 3x5cm nylon bags (52 μ m pore size) which are inserted with appropriate weights in the rumen of a sheep for 24h. These bags are removed, washed in deionised water and freeze dried and the weight of residue remaining determined. The residues and incubated samples are ground through a mill (containing a 0.5mm screen) and the starch determined on a 100mg sub-samples enzymatically using a "Megazyme" total starch assay kit (distributed by Deltagen Australia, 31 Wadhurst Drive, Boronia, Victoria Australia, 3155). All starch values measured are corrected to known standards provided in the kit. The protection of the protected carbohydrate is then calculated as the ratio of the total starch in the untreated and treated sample.

(b) Ruminal hydrogenation of unsaturated lipids.

This technique is dependent on evidence that the total long chain fatty acids passing from the abomasum is approximately equal to the intake in the diet. Hence the change in concentration of 18:2 and 18:3, gives an approximation of the degree of 5 hydrogenation. The animals are fed basal diets of chopped alfalfa hay and oats (1:1, w/w) 800g/day. The abomasal digesta is sampled via an abomasal fistula at various time periods and ca. 20mL of digesta saponified and fatty acids extracted as described for the rumen fluid incubations. The extracted fatty acids are methylated and analysed by GLC. The proportion of polyunsaturated fatty acid (eg., 18:2) in the abomasal lipids is compared 10 with a theoretical level estimated by assuming (a.) that all of the 18:2 in the lipid supplement was protected against ruminal hydrogenation; (b.) that all of the 18:2 in the basal diet was hydrogenated; and (c.) that there was no significant synthesis or degradation of the carbon skeleton of fatty acids by micro-organisms. The *in vivo* protection of these supplements is calculated using the formula:

15

$$\% \text{ protection} = \frac{\text{Actual \% 18:2 in abomasum}}{\text{Theoretical \% 18:2 in abomasum}} \times 100$$

20

As an example, a sheep receiving 400g of alfalfa hay, 400g of crushed oats and 300g of a formaldehyde treated safflower oil/casein (2:1 w/w) supplement would receive 3% of the basal diet of alfalfa and oats as fatty acids, ie., 24g, and 178g of fatty acids from the lipid supplement (corrected for glycerol moiety).

The 18:2 content of the supplementary fatty acids is 75% or 134 g. Using the above assumptions, the content of 18:2 in the abomasal fatty acids should be $134/(178 + 24) = 66\%$. If the actual 18:2 content of abomasal fatty acids is 53%, then the percentage protection $= \frac{53}{66} \times 100 = 80\%$.

3. Other Chemical Analyses

25

Moisture content of feed ingredients is determined by heating at 100°C for at least 12h. Protein content is determined by the Kjeldahl method. Formaldehyde content of supplements is determined by the method of Van Dooren J. Sci. Food Agric. (1975). 26: 1263.

30

The invention will now be described in greater detail by reference to specific to examples, which should not be construed as limiting on the scope thereof.

Examples

Example 1: Feed Supplements for the Production of Softer Fats

Feeding to lactating cows a canola/soybean blend (7:3w/w) supplement (75% protected from ruminal hydrogenation) at the rate of approximately 10% of dry matter intake, provided about 750 g fat. The fatty acid composition of the supplement and the daily intake of fatty acid per cow per day are provided below in table 1.

Table 1: Composition of Canola/Soybean Supplement (7:3 w/w) and daily intake of fatty acids

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Fatty Acid	% by Wt	g/d
18:1	51.2	345.6
18:2	28.7	193.7
18:3	10.7	72.2

Example 2: Feed Composition for the Production of Softer Fats

From the supplements described in Example 1, the following fatty acid profile was obtained from cows grazed at pasture and supplemented with the protected lipid once daily, and wherein milk was sampled after the morning milking. Control cows were grazed at pasture and were supplemented during milking with about 4kg/d of a dairy concentrate pellet containing no protected fat. The fatty acid composition of the control and fat-modified dairy products is outlined below in Table 2.

15

Table 2: Mean fatty acid profiles of control and fat-modified dairy products

Fatty Acid	Control % by wt of total fatty acids	Fat-Modified
Butyric (4:0)	5.7	5.5
Caproic (6:0)	2.7	2.5
Caprylic (8:0)	2.9	1.3
Capric (10:0)	2.8	2.3
Lauric (12:0)	3.3	2.3
Myristic (14:0)	10.0	6.7
Palmitic (16:0)	25.9	15.5

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Stearic (18:0)	11.7	14.3
Oleic (18:1)	22.8	35.3
Linoleic (18:2)	1.5	6.9
Linolenic (18:3)	0.7	2.2

Example 3: Feed supplement for the production of milk fat enriched with C20 and C22 n-3 fatty acids

Feeding lactating cows a rumen protected tuna oil-soybean lipid/protein (sunflower meal) (23:67:10; w/w/w) supplement (75% rumen protection *in vitro*) at the rate of approximately 2.2Kg/h/day, the following fatty acid profile was obtained. Control cows were grazed at pasture and were supplemented during milking with about 4kg/d of a dairy concentrate pellet containing no protected fat. The fatty acid composition of the control and fat-modified dairy products is outlined below in Table 3:

10

Table 3. Fatty acid profile of control and C20, C22 (n-3) enriched milk fat

Fatty acid	Control milk fat	n-3 enriched milk fat
< C14:0	13.7	7.6
C14:0	11.0	8.7
C16:0	31.1	23.6
C16:1	1.5	1.1
C18:0	10.8	11.9
C18:1	23.6	27.6
C18:2	2.4	6.2
C18:3	0.2	1.3
C20:5	Nd	0.5
C22:6	Nd	1.0

Nd=not detectable

Note the significant increase in the proportion of the C20:5 and C22:6 fatty acids in milk from cows consuming protected tuna oil supplement.

Example 4: Feed supplements for the production of milk fat enriched with C18 n-3 fatty acids

Feeding lactating cows a mixture containing 90 parts of rumen protected linseed oil-soybean lipid (3:7w/w; 80% rumen protection *in vitro*) and 10 parts of rumen protected sunflower meal protein (60% rumen protection *in vitro*) at the rate of approximately 1.5Kg/h/day, the following fatty acid profile was obtained. Control cows were grazed at pasture and were supplemented during milking with about 4kg/d of a dairy concentrate pellet containing no protected fat. The fatty acid composition of the control and fat-modified dairy products is outlined below in Table 4:-

10

Table 4. Fatty acid profile of control and C18 (n-3) enriched milk fat

Fatty acid	Control milk fat	n-3 enriched milk fat
C8:0	2.0	2.0
C10:0	2.5	2.5
C12:0	2.7	2.6
C14:0	10.6	8.3
C16:0	30	18.6
C16:1	0.4	0.4
C18:0	8.2	11.5
C18:1 cis	24.5	24.0
C18:1 trans	2.2	2.9
C18:2	2.6	8.2
C18:3	0.7	8.6

Note the significant increase in the proportion of the C18 n-3 fatty acids content in milk fat from cows consuming protected linseed oil supplement.

15

Example 5: Feed supplement for the production of milk fat enriched in C18:1 cis mono-unsaturated fat

Feeding lactating cows a rumen protected sunola oil/casein supplement (1:1; w/w) (80% rumen protection *in vitro*) containing 10% protected protein (sunflower meal) supplement at the rate of 3Kg/h/d, the following fatty acid profile was obtained. As per 20 the examples outlined above, control cows were grazed at pasture and were supplemented during milking with about 4kg/d of a dairy concentrate pellet containing no protected fat.

The fatty acid composition of the control and fat-modified dairy products is outlined below in Table 5:-

Table 5. Fatty acid profile of control and C18:1 *cis* enriched milk fat

5

Fatty acid	Control milk fat	n-3 enriched milk fat
C8:0	2.5	2.0
C10:0	3.0	1.7
C12:0	3.0	2.0
C14:0	10.6	7.5
C16:0	31.7	18.2
C16:1	1.6	0.6
C18:0	13.4	13.2
C18:1 <i>cis</i>	21.6	43.2
C18:1 <i>trans</i>	2.1	1.9
C18:2	2.0	3.6
C18:3	0.5	0.7

Note the significant increase in the C18 *cis* monounsaturated fatty acid in milk fat from cows fed the protected sunola oil supplement.

10 The following table demonstrates that the feeding of rumen protected lipid supplements significantly increases the proportion of fats that is soft at different temperatures.

Table 6. The melting characteristics of milk fat from cows at Pasture or supplemented with rumen protected lipids

15

Melting Characteristics	Pasture	Linseed-Soybean lipid	Tuna oil-Soybean lipid
Liquid at 5° C (%)	35.3	65.1	55.4
Liquid at 20° C (%)	68.3	90.4	86.5

Example 6: Feed supplements for the production of milk fat enriched with C18 conjugated linoleic acid (CLA's).

Feeding to lactating goats a rumen protected CLA/casein supplement (1:1; w/w) (70% rumen protection *in vitro*) at the rate of 80g /h/d produced the following fatty acid profile in milk, the following fatty acid profile was obtained. Control goats were fed 2.4 kg/d of lucerne chaff/oat grain (60:40 w/w). The fatty acid composition of the control and fat-modified dairy products is outlined below in Table 7:-

Table 7. Fatty acid profile of control and CLA enriched milk fat from goats

10

Fatty acid	Control milk fat	n-3 enriched milk fat
< C14:0	11.1	6.4
C14:0	8.3	6.7
C16:0	23.6	22.1
C18:0	14.7	23.5
C18:1	26.1	22.6
C18:2	2.2	2.7
C18:3	0.7	0.7
CLA 9c, 11t	0.6	2.2
CLA 10t, 12c	nd	1.9

Nd=Not detected

Note the significant increase in the two major CLA isomers (*9 cis, 11 trans; 10 trans, 12 cis*) in milk fat from goats fed the protected CLA supplement.

Example 7: Feed Composition for the Production of Harder Fats

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In this example, the proportion of C18:0 increased and there was a decrease in C18:1, thereby resulting in a substantial increase in both the melting point of milk fat and its hardness. This change is outlined in Figure 2, and again illustrates the role of protected lipids in altering the proportions of fatty acids in milk.

20

The feeding regime used to induce the changes in Figure 2 comprised a basal ration of lucerne hay and oat grain (1:1, w/w) supplemented with varying levels of protected cotton seed ranging from 0-80% which replaced a canola soybean (80:20, w/w) supplement to provide 110 grams of protected fat per day.

Example 8: Protected Lipid Preparation

Cottonseed was coarsely comminuted in a hammer mill and mixed with ethoxyquin (150ppm on an oil basis). This material was then mixed with water to produce a slurry and, after emulsification of the oil and protein in a colloid stone mill, the 5 caustic soda was added to solubilise the oilseed protein. The protein constituents of the homogenised oil seed were cross-linked with 37% (w/v) formaldehyde at the rate of approximately 1.5-3g formaldehyde per 100g crude portion to form a gel which was then dried in a pneumatic drier with an average hot air temperature of 300°C to complete the reaction and produced a protected lipid that was 60-90% resistant to metabolism in the 10 rumen *in vitro*.

(a) Protected Canola Lipid

Canola lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 15 2.5g formaldehyde per 100g crude portion producing a supplement that was 75% resistant to metabolism in the rumen *in vitro*.

(b) Protected Cotton Lipid

Cotton lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 20 3.0g formaldehyde per 100g crude portion producing a supplement that was 80% resistant to metabolism in the rumen *in vitro*.

(c) Protected Cotton - Tallow Lipid

Cotton-tallow lipid was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 25 2.5g formaldehyde per 100g crude portion producing a supplement that was 80% resistant to metabolism in the rumen *in vitro*.

(d) Protected Fish Oil - Soybean Lipid

Soybean-fish oil was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 30 2.5g formaldehyde per 100g crude portion producing a supplement that was 75% resistant to metabolism in the rumen *in vitro*.

(e) Protected Linseed Oil - Soybean Lipid

Soybean-Linseed oil was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 35 2.5g formaldehyde per 100g crude portion producing a supplement that was 80% resistant to metabolism in the rumen *in vitro*.

(f) Protected Sunola - Soybean Lipid

Soybean-Sunola oil was emulsified with protein, and the protein constituents of the homogenised oil seed were cross-linked with formaldehyde at a rate of approximately 2.5g formaldehyde per 100g crude portion producing a supplement that was 75% resistant to metabolism in the rumen *in vitro*.

(g) Protected Conjugated linoleic acids (CLA)

An oil containing 60% conjugated linoleic acid was emulsified with casein, and the protein constituents of the homogenised oil were cross-linked with formaldehyde at a rate of approximately 2.5g formaldehyde per 100g crude portion, producing a supplement that was 70% resistant to metabolism in the rumen *in vitro*.

Example 9: Protection of Protein Supplements

Protected protein was prepared by spraying 37% (W/V) formaldehyde at the rate of between 0.05 and 0.8g formaldehyde per 100g crude protein into a rapid mixing device containing milled oil seed meal (38% crude protein). This material was then transferred to sealed storage for 10 days to give a supplement 50-70% resistant to proteolysis in the rumen.

(a) Protected Sunflower Protein

Protected sunflower protein was prepared by reacting approximately 0.7g formaldehyde per 100g with milled sunflower seed meal (38% crude protein, 2% crude lipid), producing a supplement 65% resistant to proteolysis in the rumen.

(b) Protected Canola Protein

Protected canola protein was prepared by reacting approximately 0.5g formaldehyde per 100g with milled canola seed meal (38% crude protein, 2% crude lipid), producing a supplement 70% resistant to proteolysis in the rumen.

(c) Protected Lupin Protein

Protected lupin protein was prepared by reacting approximately 0.6g formaldehyde per 100g with milled lupin seed meal (38% crude protein, 5% crude lipid), producing a supplement 65% resistant to proteolysis in the rumen.

(d) Protected Cottonseed Protein

Protected cottonseed protein was prepared by reacting approximately 0.3g formaldehyde per 100g with milled cottonseed seed meal (38% crude protein, 2% crude lipid), producing a supplement 75% resistant to proteolysis in the rumen.

Example 10: Protection of Carbohydrate Supplements

Grain was coarsely comminuted in a hammer mill to a particle size of approximately 2.5mm or smaller. Protected carbohydrate was then prepared by spraying 37% (W/V) formaldehyde at the rate of between 0.1 and 3.0 grams formaldehyde per 5 100g crude carbohydrate into a rapid mixing device containing milled concentrate. This material was then transferred to sealed storage for 10 days to give a protected carbohydrate supplement 30-80% resistant to degradation in the rumen.

(a) Protected Wheat Carbohydrate

Protected wheat carbohydrate was prepared by reacting approximately 1.2g 10 formaldehyde per 100g with milled wheat, producing a supplement 65% resistant to degradation in the rumen.

(b) Protected Barley Carbohydrate

Protected barley carbohydrate was prepared by reacting approximately 1.4g 15 formaldehyde per 100g with milled barley, producing a supplement 70% resistant to degradation in the rumen.

Industrial Applicability

The present invention makes use of nutritional materials protected against rumen degradation, but offers the possibility of altering the fatty acid profile of milk produced from female ruminant livestock. In particular, it describes feed supplements which 20 produce milk with a desired fatty acid composition and are useful in producing products with a range of melting profiles. Practise of this invention can be expected to offer economic benefits irrespective of the type of animal in question.

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Claims

1. A method for altering the fatty acid profile of milk from female ruminant livestock to comprise at least one of the following types and proportions of fatty acids in said milk: C18:1 *cis* (25-45%w/w); C18:2 (4-15%w/w); C18:3 (1-8%w/w); C20:5 and C22:6 omega fatty acid (1-3%w/w), or a combination thereof, wherein said method comprises feeding to the female ruminant livestock, protected lipid such that about 60 to about 90% of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally, and wherein said protected lipid is produced by the emulsification of lipid with protein and the mixing of between about 1.5 grams and about 3 grams of formaldehyde per 100 grams crude portion of said emulsified lipid-protein complex.

2. The method of claim 1, wherein said fatty acid profile comprises at least one of: C18:1 *cis* (30-45%w/w); C18:2 (6-10%w/w); C18:3 (2-4%w/w); C20:5 and C22:6 omega fatty acid (1-2%w/w), or a combination thereof.

3. The method of claim 1 or 2, wherein said C18:2 further includes conjugated isomers (0.5 to 5%w/w).

4. A method for altering the fatty acid profile of milk from female ruminant livestock to have at least one of the following types and/or proportions of fatty acids in said milk: C16:0 *cis* (25-35%w/w), C18:0 (20-30%w/w) and C18:1 (20-25%w/w), wherein said method comprises feeding to the ruminant livestock, protected lipid having said desired proportions and/or types of fatty acids, such that about 60 to about 90% of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90% of said protected lipid available for digestion post-ruminally, and wherein said protected lipid is produced by the emulsification of lipid with protein and the mixing of between about 1.5 grams and about 3 grams of formaldehyde per 100 grams crude portion of said emulsified lipid-protein complex.

5. The method of claim 4, wherein said fatty acid profile comprises at least one of: C16:0 *cis* (28-35%w/w), C18:0 (25-30%w/w) and C18:1 (22-25%w/w), or a combination thereof.

6. The method according to any one of claims 1-5, wherein about 75 to about 90% of protected lipid is capable of passing undegraded through the rumen.

7. The method according to any one of claims 1-6, wherein the protected lipid is produced by the reaction with between about 2.0 grams and about 2.6 grams of formaldehyde per 100 grams crude portion.

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8. The method according to any one of claims 1 to 7, wherein the source of lipid is selected from the group consisting of: soybean, cotton, lupin, peanut, sunflower, sunola, canola, sesame seed, copra and coconut, palm kernels, linseed, casein, butterfat, yellow grease, lard, fish oils, tung oil, tallow, and oil lipid products derived from oil sources by chemical/biological processes, or a combination thereof.

9. The method according to claim 8, wherein the source of oil lipid product is conjugated linoleic acid or chemical forms thereof.

10. The method according to claim 9, wherein the source of lipid is derived by chemical/biological processes, or a combination thereof.

11. The method according to any one of claims 1 to 10, further comprising simultaneously feeding to the ruminant livestock protected protein, such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein available for digestion post-ruminally.

12. The method according to any one of claims 1 to 10, further comprising simultaneously feeding to the ruminant livestock protected carbohydrate such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

13. The method according to any one of claims 1 to 10 further comprising simultaneously feeding to the ruminant livestock: (i) protected protein, such that such that about 60 to about 80% of said protected protein is capable of passing through the rumen undigested leaving about 60 to about 80% of said protected protein is available for digestion post-ruminally, and (ii) protected carbohydrate, such that about 30 to about 80% of said protected carbohydrate is capable of passing through the rumen undigested leaving about 30 to about 80% of said protected carbohydrate available for digestion post-ruminally.

14. A method according to any one of claims 11-13, wherein the source of protein and/or carbohydrate is plant and includes any one of, or a combination of barley, corn, oats, wheat, rice, millet, triticale, rye, and sorghum, soybean, cotton, lupin, peanut, sunflower, sunola, canola, sesame seed, copra and coconut, palm kernels and linseed.

15. The method according to any one of claims 1 to 14, further comprising, feeding to the ruminant livestock any other source of processed or unprocessed feedstuff.

16. The method according to any one of claims 13 to 15, wherein the protected carbohydrate, protected protein and/or protected lipid is included in the ration at about 10-45% during the lactation phase.

5 17. Milk fat obtained from a ruminant fed according to the method of any one of claims 1 to 16.

18. The milk fat of claim 17, wherein said milk fat is comprised of nutritionally desirable soft fats, including n-3 and n-6 essential fatty acids, conjugated linoleic acid and C₂₀ and C₂₂ polyenoic fatty acids.

19. The milk fat of claim 18, wherein said milk fat is comprised of hard fats.

10 20. The milk fat of any one of claims 17 to 19, wherein said milk fat is used in the production of milk based products.

21. The milk fat of any one of claims 17 to 20, wherein said milk based products include: milk, butter, cheese, yoghurt, chocolate or infant formula.

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(54) Title: **FEED SUPPLEMENT FOR ALTERING MILK FAT PROFILE**

(57) Abstract: The present invention provides a method for altering the fatty acid profile of milk from female ruminant livestock to have desired proportions and/or types of fatty acids, wherein said method comprises feeding to the ruminant livestock, protected lipid having said desired proportions of fatty acids, such that about 60 to about 90 % of said protected lipid is capable of passing through the rumen undigested leaving about 60 to about 90 % of said protected lipid available for digestion post-ruminally.

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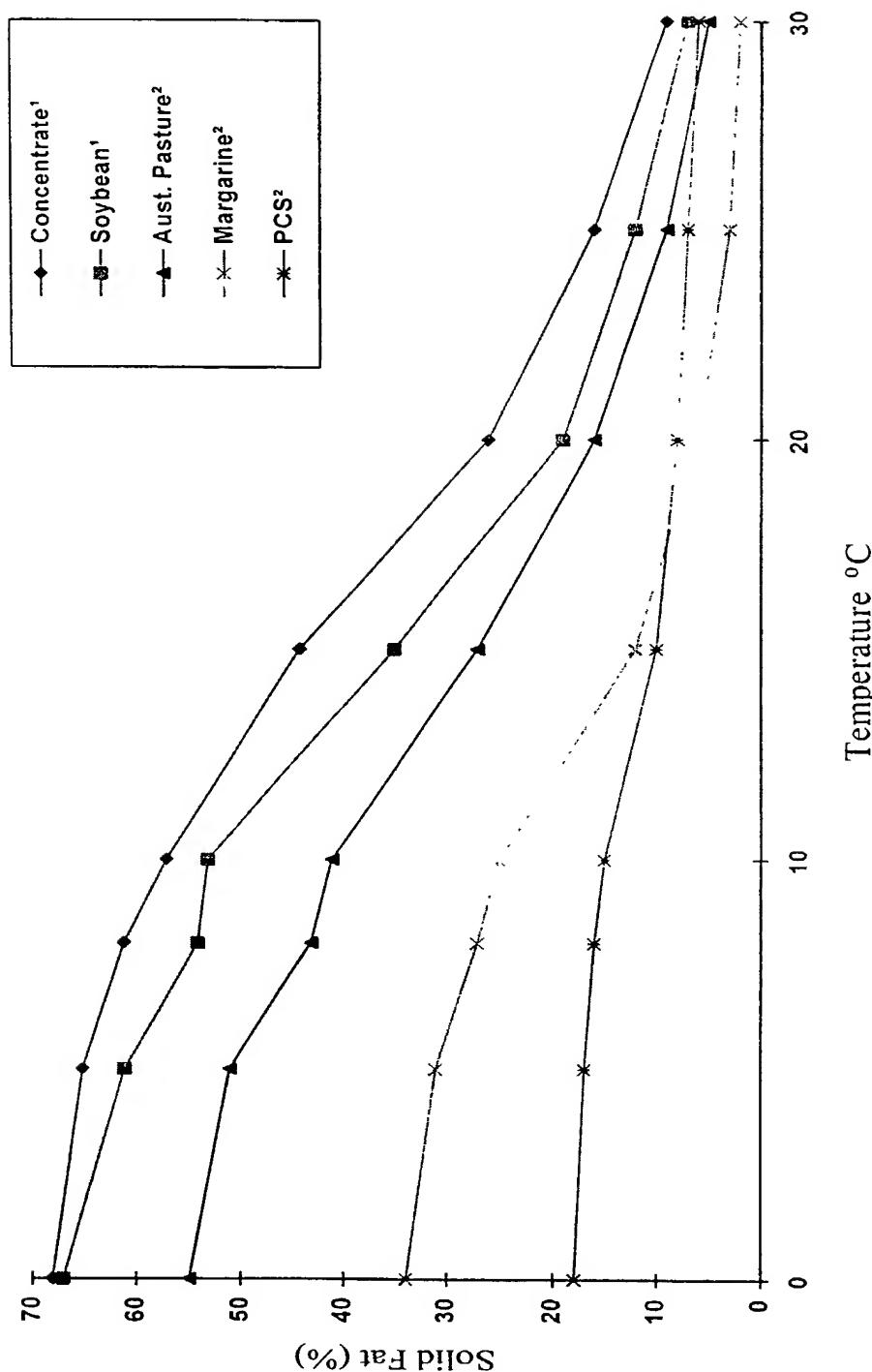


Figure 1

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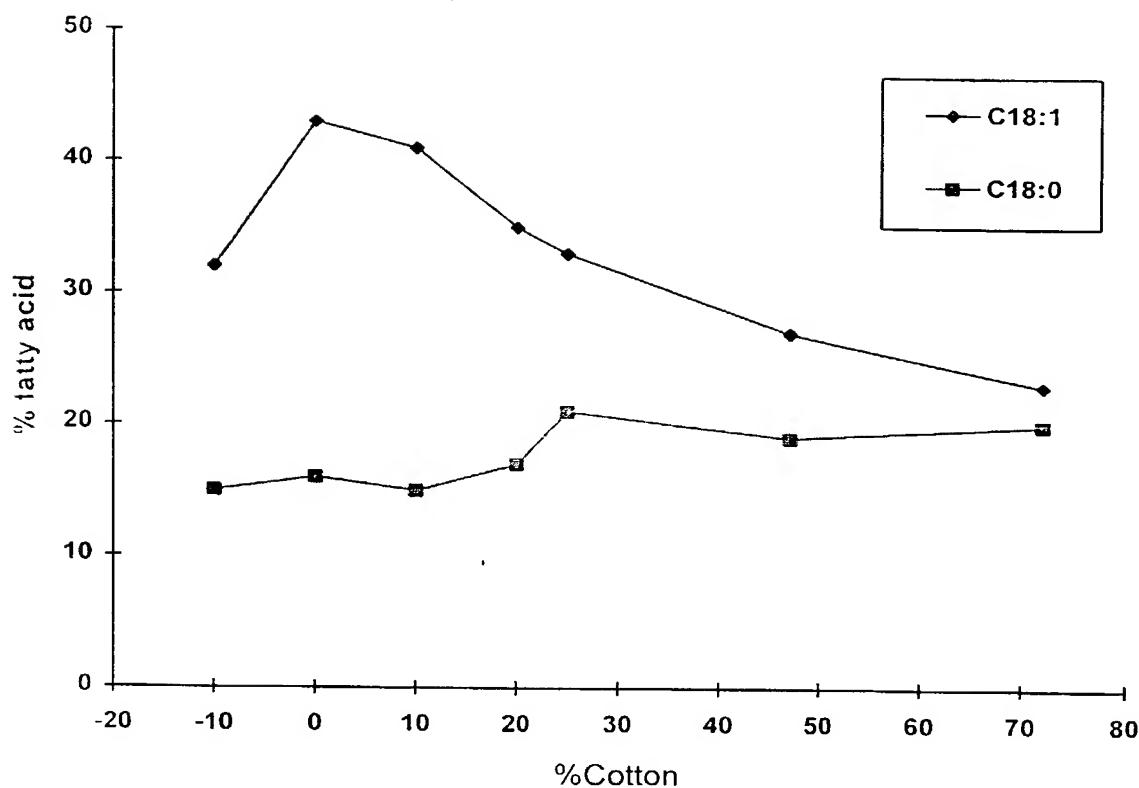


Figure 2

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
 (Includes Reference to PCT International Application(s))

Attorney's Docket Number
 51454-013

As below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

FEED SUPPLEMENT FOR ALTERING MILK FAT PROFILE

the specification of which.

is attached hereto

was filed as United States application Serial No. _____
on _____

and was amended on February 13, 2002 (if applicable).

was filed as PCT international application Number PCT/AU00/00953
on August 11, 2000

and was amended under PCT Article 19 on December 15, 2000 (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose information which is known to me to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or Section 365(b) of any foreign and/or international application(s) for patent or inventor's certificate or Section 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (If PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/AU00/00953	August 11, 2000	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
AUSTRALIA	PQ2218	August 13, 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below.

PRIOR PROVISIONAL APPLICATION(S):

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check One)		
U.S. Application Number	U.S. Filing Date	Patented	Pending	Abandoned

PCT APPLICATIONS DESIGNATING THE U.S.

PCT Application No.	PCT Filing Date	U.S. Serial Numbers Assigned (if any)
PCT/AU00/00953	August 11, 2000	

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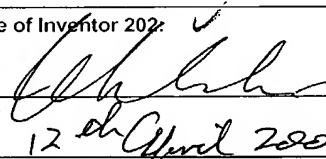
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	Signature of Inventor 201: ✓ <i>Suresh Kumar</i>	Signature of Inventor 202: ✓	Signature of Inventor 203: <i>Suresh</i>	
Date 9. 4. 02	Date ✓	Date 9. 4. 02		

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Signature of Inventor 201:	Signature of Inventor 202:  12 th April 2002	Signature of Inventor 203:
Date	Date	Date